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**IN THE UNITED STATES DISTRICT COURT**
**FOR THE DISTRICT OF NEW JERSEY**

| | | |
|---|---|---|
| CARGILL, INCORPORATED | ) | |
| | ) | |
| Plaintiff, | ) | |
| | ) | |
| v. | ) | Civil Action No. |
| | ) | |
| NANTONG FOREIGN TRADE MEDICINES AND HEALTH PRODUCTS CO., LTD., DNP INTERNATIONAL, INC., TIANCHENG INTERNATIONAL, INC. (USA), HYGIEIA HEALTH CO., LTD., TSI HEALTH SCIENCES, INC. (USA), and ETHICAL NATURALS, INC. | ) | |
| | ) | |
| Defendants. | ) | |

**COMPLAINT AND JURY DEMAND**

Plaintiff, Cargill, Incorporated ("Cargill"), with its principal place of business at 15047 McGinty Road West, Wayzata, Minnesota 55391, by its attorneys, Mayer Brown LLP, alleges for its Complaint against Defendants Nantong Foreign Trade Medicines and Health Products Co., Ltd., with its principal place of business at 6/F Commercial Building, 15 Middle Quingnian Rd., Nantong, Jiangsu, China 226006, DNP International, Inc., with its principal place of business at 12802 Leffingwell Ave., Bldg. E, Santa Fe Springs, CA 90670, Tiancheng International, Inc. (USA), with its principal place of business at 2851 E. Philadelphia St., Ontario, CA 91761, Hygieia Health Co., Ltd., with its principal place of business at Building # 54, 5/F, 1089 Qinzhou Road (N), Shanghai, China 200233, TSI Health Sciences, Inc. (USA),

with its principal place of business at 7168 Expressway, Missoula, Montana 59808, and Ethical Naturals, Inc., with its principal place of business at 330 Sir Francis Drake Blvd., Suite H, San Anselmo, CA 94960 (collectively, "Defendants") on knowledge as to itself and its own acts and upon information and belief as to all other matters, as follows:

## SUMMARY OF COMPLAINT

1. This is an action for patent infringement pursuant to the patent laws of the United States, 35 U.S.C. §100, et seq., arising out of Defendants' willful and deliberate infringement of the patent described below.

2. United States Letters Patent No. 7,049,433 ("the '433 patent") was issued to Weiyu Fan, John A. Bohlmann, James R. Trinkle, James Donald Steinke, Ki-Oh Hwang and Joseph P. Henning on May 23, 2006. Cargill is the sole owner, by valid assignment, of all right, title and interest in and to the '433 patent. The patent describes and claims methods for producing fungal-derived glucosamine compositions from chitin present in fungal biomass. Glucosamine is used as a nutraceutical supplement and can be used as a food additive.

3. Cargill provided notice of its patent to Defendants. The notice explicitly informed the recipients that Cargill owned the rights to the '433 patent and that certain of Defendants' activities and products were covered by the patent.

4. However, Defendants have continued to refuse to recognize the '433 patent and have willfully and deliberately infringed the '433 patent by, among other things, using, importing, manufacturing, offering for sale, selling, distributing and/or promoting non-shellfish glucosamine and products containing same in a manner designed to directly infringe, contributorily infringe and/or induce infringement of the '433 patent.

## JURISDICTION AND VENUE

5. This is an action for patent infringement arising under the patent laws of the United States, Title 35, United States Code. This Court has jurisdiction over this action pursuant to 28 U.S.C. §§ 1331 and 1338(a).

6. Venue is proper in this District pursuant to 28 U.S.C. § 1391(b) and (c), and 28 U.S.C. § 1400(b).

## THE PARTIES

7. Plaintiff Cargill is a privately owned Delaware corporation with its principal place of business at 15047 McGinty Road West, Wayzata, Minnesota 55391. Cargill is an international provider of food, agricultural and risk management products and services employing about 158,000 employees. Among other businesses, Cargill develops, manufactures, and markets science-based, health promoting ingredients and ingredient systems for makers of food, dietary and pharmaceutical products. Cargill is the assignee of the '433 patent issued to Weiyu Fan, John A. Bohlmann, James R. Trinkle, James Donald Steinke, Ki-Oh Hwang and Joseph P. Henning (the "Inventors").

8. Upon information and belief, defendant Nantong Foreign Trade Medicines & Health Products Co., Ltd. ("NFT"), is a corporation organized under the laws of China, and has a principal place of business at 6/F Commercial Building, 15 Middle Quingnian Rd., Nantong, Jiangsu, China 226006. Upon information and belief, NFT manufactures non-shellfish glucosamine, which NFT imports and/or uses, offers for sale, sells for importation into, and sale after importation in, the United States. Specifically, on information and belief, NFT sells non-shellfish glucosamine for importation, and/or provides samples for importation, to DNP International, Inc. and Tiancheng International, Inc. (USA), among others, for sale in this judicial

district and elsewhere throughout the United States. NFT has marketed its products for sale in this district at least through its attendance at the Supply Side East Trade Show in April, 2008.

9. Upon information and belief, defendant DNP International, Inc. ("DNP"), is a corporation organized under the laws of California and has a principal place of business at 12802 Leffingwell Ave., Bldg. E, Santa Fe Springs, CA 90670. Upon information and belief, DNP imports, distributes, and/or uses, offers for sale and sells non-shellfish glucosamine obtained from NFT both directly and via Tiancheng International, Inc. (USA) after importation in the United States. DNP has marketed its products for sale in this district at least through its attendance at the Supply Side East Trade Show in April, 2008.

10. Upon information and belief, defendant Tiancheng International, Inc. (USA) ("Tiancheng (USA)"), is a corporation organized under the laws of California and has a principal place of business at 2851 E Philadelphia St., Ontario, CA 91761-8553. Upon information and belief, Tiancheng (USA) imports, distributes, and/or uses, offers for sale and sells non-shellfish glucosamine obtained from NFT after importation in the United States. Tiancheng has marketed its products for sale in this district at least through its attendance at the Supply Side East Trade Show in April, 2008.

11. Upon information and belief, defendant Hygieia Health Co., Ltd. ("Hygieia"), is a corporation organized under the laws of Hong Kong with its global headquarters at Unit A/6F Two Chinachem Plaza, 68 Connaught Road, Central, Hong Kong, and has its principal place of business at Building # 54, 5/F 1089 Qinzhou Road (N), Shanghai, China 200233. Upon information and belief, Hygieia manufactures non-shellfish glucosamine, which Hygieia imports and/or uses, offers for sale, sells for importation into, and sale after importation in, the United States. Specifically, on information and belief, Hygieia sells non-shellfish glucosamine for

importation to its affiliated party, TSI Health Sciences, Inc. (USA), for sale in this judicial district and elsewhere throughout the United States. On information and belief, Hygieia is the alter ego of, and part of a same corporate entity with TSI Health Sciences, Inc., which also acts as Hygieia's agent in selling Hygieia's products in the United States.

12. Upon information and belief, defendant TSI Health Sciences, Inc. (USA) ("TSI (USA)"), an affiliate of Hygieia, is a corporation organized under the laws of Montana and has a principal place of business at 7168 Expressway, Missoula, Montana 59808-8587. Upon information and belief, TSI (USA) imports, distributes, and/or uses, offers for sale and sells non-shellfish glucosamine after importation in the United States. TSI (USA) has marketed its products for sale in this district at least through its attendance at the Supply Side East Trade Show in April, 2008.

13. Upon information and belief, defendant Ethical Naturals, Inc. ("ENI"), is a corporation organized under the laws of California and has a principal place of business at 330 Sir Francis Drake Blvd., Suite H, San Anselmo, CA 94960. Upon information and belief, ENI imports, distributes, and/or uses, offers for sale and sells non-shellfish glucosamine after importation in the United States. ENI has marketed its products for sale in this district at least through its attendance at the Supply Side East Trade Show in April, 2008.

## THE PATENT

14. On May 23, 2006, United States Letters Patent No. 7,049,433, entitled "Glucosamine and Method of Making Glucosamine From Microbial Biomass" was duly and legally issued to the Inventors and thereafter assigned to Cargill. A copy of this patent is attached hereto as Exhibit 1. The '433 patent describes and claims methods for producing fungal-derived non-shellfish glucosamine compositions from chitin present in fungal biomass.

Cargill invented this process for making non-shellfish derived glucosamine and sells this product under the trade name Regenasure®.

**CLAIM FOR RELIEF**
**(Infringement of the '433 Patent)**

15. Cargill repeats and realleges the allegation of paragraphs 1 through 14 as if fully set forth herein.

16. Defendants are engaged in the importation, manufacture, distribution and/or sale of non-shellfish glucosamine and products containing same in this judicial district and elsewhere in the United States. The accused non-shellfish glucosamine and products containing same infringe, or are made or produced under, or by means of, a process covered by claims 1-10 of the '433 patent pursuant to 35 U.S.C. § 271(g).

17. Pursuant to 35 U.S.C. § 295, this Court may presume that a product was made by Cargill's patented methods where there is a substantial likelihood that it was so made and Cargill has made reasonable efforts to determine the process actually used. Here, there is substantial likelihood that Cargill's methods were used by Defendants' manufacture of non-shellfish glucosamine and products containing same. Testing of glucosamine samples obtained from Defendants indicate the presence of citric acid. The presence of a measurable amount of citric acid in a given sample of glucosamine strongly suggests that the glucosamine was obtained from fungal biomass used in citric acid fermentation. Further, the results of other analyses conducted by Cargill have resulted in findings that there is no commercially feasible means of manufacturing glucosamine from fungal chitin using acid hydrolysis which does not use the process claimed in the '433 patent. Thus, based on the above and other evidence, there is a substantial likelihood that the glucosamine sampled from Defendants was made by a process that infringes the methods claimed in the '433 patent.

18. Cargill has made reasonable efforts to determine the process actually used by each Defendant. Among other things, Cargill has requested Defendants to identify the methods used in its facilities. NFT has admitted to Cargill that its process infringes on the process described in the '433 patent. Counsel for Hygieia has refused to provide any detailed information concerning the process used by Hygieia. Counsel for ENI has provided limited information to identify the process actually used by ENI, which information is insufficient for Cargill to conclude that the process used by ENI is not the process identified in the '433 patent. In light of the above, Cargill has made the required reasonable efforts under 35 U.S.C. § 295.

19. On information and belief, NFT manufactures non-shellfish glucosamine outside the United States, marketed by NFT as Vegan D-Glucosamine HCL and Vegan D-Glucosamine sulfate (2KCL), which NFT then imports, uses, offers for sale, sells for importation, and/or sells in the United States after importation, and which infringes claims 1-10 of the '433 patent.

20. On information and belief, DNP imports, uses, offers for sale, distributes, and/or sells non-shellfish derived glucosamine in the United States after importation, which infringes claims 1-10 of the '433 patent.

21. On information and belief, Tiancheng (USA) imports, uses, offers for sale, distributes, and/or sells non-shellfish glucosamine in the United States after importation, which infringes claims 1-10 of the '433 patent.

22. On information and belief, Hygieia manufactures non-shellfish glucosamine outside the United States, marketed by Hygieia as GlucosaGreen®, which Hygieia then imports, uses, offers for sale, sells for importation, and/or sells in the United States after importation, and which infringes claims 1-10 of the '433 patent.

23. On information and belief, TSI (USA) imports, uses, offers for sale, distributes, and/or sells non-shellfish derived glucosamine in the United States after importation, including GlucosaGreen® purchased from Hygieia in China, which infringes claims 1-10 of the '433 patent.

24. On information and belief, ENI imports, uses, offers for sale, distributes, and/or sells non-shellfish glucosamine in the United States after importation, which ENI markets as GreenGrown® Gluscosamine, and which infringes claims 1-10 of the '433 patent.

25. Defendants have imported, used, manufactured, distributed, sold and/or offered to sell its non-shellfish derived glucosamine and products containing same with the knowledge that those products infringe, or are made or produced under, or by means of, a process covered by the '433 patent. Defendants' non-shellfish derived glucosamine products are not a staple article or commodity of commerce suitable for substantial noninfringing use.

26. By virtue of these activities, Defendants have been directly infringing, contributorily infringing and/or inducing the infringement of the '433 patent.

27. Defendants have received express notice of the '433 patent and/or had prior knowledge of that patent prior to the filing of this complaint. To date, Defendants continue to directly infringe, contribute to and/or induce infringement of the '433 patent in violation of 35 U.S.C. § 271.

28. Defendants' actions have been willful and deliberate, entitling Cargill to increased damages under 35 U.S.C. § 284 and making this an exceptional case within the meaning of 35 U.S.C. § 285.

29. The foregoing acts of patent infringement by Defendants has caused, and unless enjoined by this Court, will continue to cause, immediate and irreparable injury and damage to Cargill.

## PRAYER FOR RELIEF

WHEREFORE, Cargill prays for the following relief and judgment from this Court:

A. Finding that the '433 patent has been infringed by the Defendants, as alleged herein;

B. Awarding damages adequate to compensate Cargill for Defendants' infringement, but not less than a reasonable royalty for the use made of the claimed invention by each Defendant, together with interest, including pre-judgment interest, and costs as fixed by the Court;

C. Awarding Cargill an accounting of all Defendants' sales of products found to infringe;

D. Finding that Defendants' infringements have been willful and deliberate;

E. Awarding Cargill increased damages and attorneys' fees pursuant to 35 U.S.C. § 284 and § 285 because of the willful and deliberate nature of Defendants' infringement;

F. Permanently enjoining Defendants and its officers, agents, servants, employees and affiliates, as well as all others in active concert or participation with them as any of the foregoing, from inducing or contributing to the infringement of the '433 patent;

G. Awarding Cargill such other and further relief as this Court may deem just and proper.

## JURY DEMAND

Plaintiff Cargill hereby demands a trial by jury on all issues so triable in this matter.

Dated: January 28, 2009

Respectfully submitted,

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# EXHIBIT 1

US007049433B2

(12) **United States Patent**
**Fan et al.**

(10) **Patent No.: US 7,049,433 B2**
(45) **Date of Patent: *May 23, 2006**

(54) **GLUCOSAMINE AND METHOD OF MAKING GLUCOSAMINE FROM MICROBIAL BIOMASS**

(75) Inventors: **Weiyu Fan**, Minnetonka, MN (US); **John A. Bohlmann**, Ottumwa, IA (US); **James R. Trinkle**, Bussey, IA (US); **James Donald Steinke**, Oskaloosa, IA (US); **Ki-Oh Hwang**, Oskaloosa, IA (US); **Joseph P. Henning**, Eddyville, IA (US)

(73) Assignee: **Cargill, Incorporated**, Wayzata, MN (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 158 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/326,549**

(22) Filed: **Dec. 19, 2002**

(65) **Prior Publication Data**

US 2003/0148998 A1 Aug. 7, 2003

**Related U.S. Application Data**

(63) Continuation of application No. 09/785,695, filed on Feb. 16, 2001, now abandoned.

(51) **Int. Cl.**
***C08B 37/00*** (2006.01)
***C07H 5/04*** (2006.01)
***A61K 31/70*** (2006.01)

(52) **U.S. Cl.** ..... **536/55.2**; 536/55.3; 514/62

(58) **Field of Classification Search** ..... 536/55.2, 536/55.3; 514/62

See application file for complete search history.

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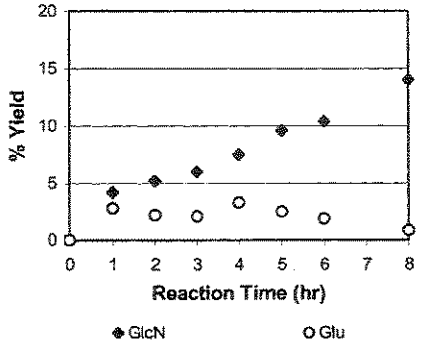
*Primary Examiner*—Shengjun Wang
(74) *Attorney, Agent, or Firm*—Klarquist Sparkman, LLP

(57) **ABSTRACT**

Glucosamine suitable for human or animal consumption is disclosed. The glucosamine is derived from microbial biomass containing chitin. Suitable starting materials include substantially uniform microbial fungal sources, such as fungal sources derived from *Aspergillus* sp., *Penicillium* sp., *Mucor* sp. and combinations thereof. Methods of producing glucosamine by acid hydrolysis of fermented fungal biomass are also disclosed.

**13 Claims, 3 Drawing Sheets**

**% Yield of Glucosamine and Glucose at Hydrolysis Conditions**



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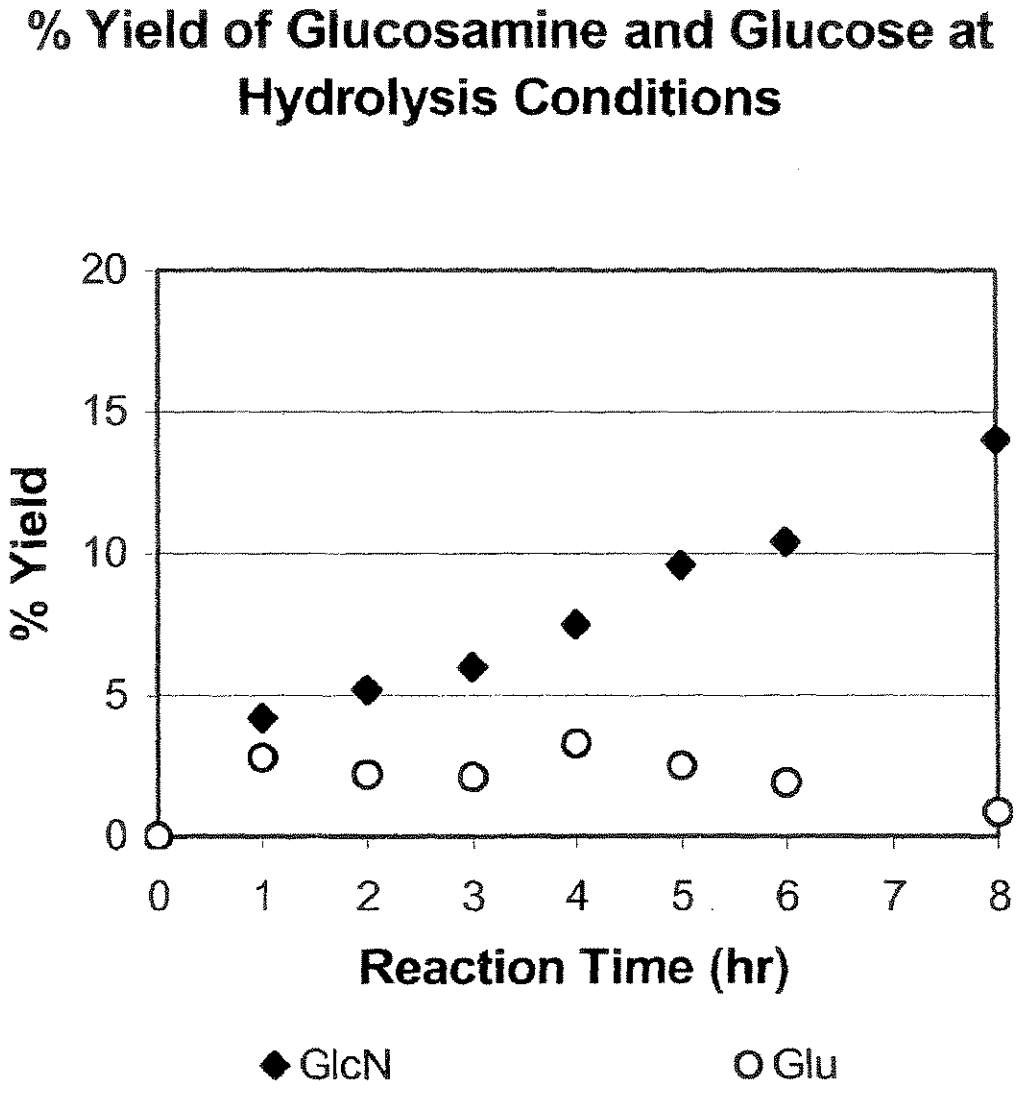

% Yield of Glucosamine and Glucose at Hydrolysis Conditions
20
15
10
5
0
% Yield
0
1
2
3
4
5
6
7
8
Reaction Time (hr)
GlcN
Glu

FIG. 1

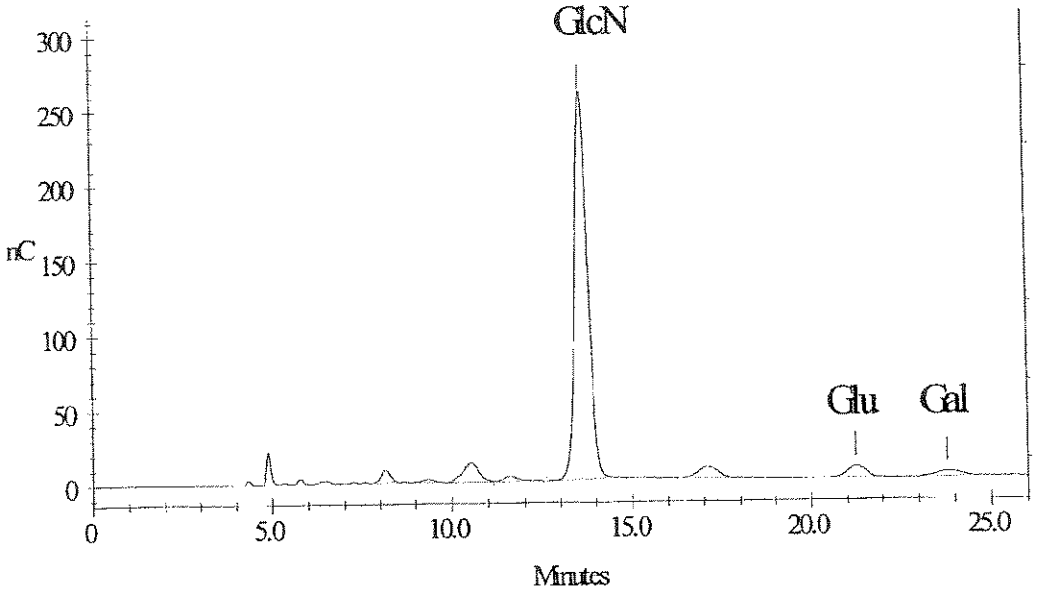

GlcN
Glu
Gal
nC
300
250
200
150
100
50
0
0
5.0
10.0
15.0
20.0
25.0
Minutes

FIG. 2

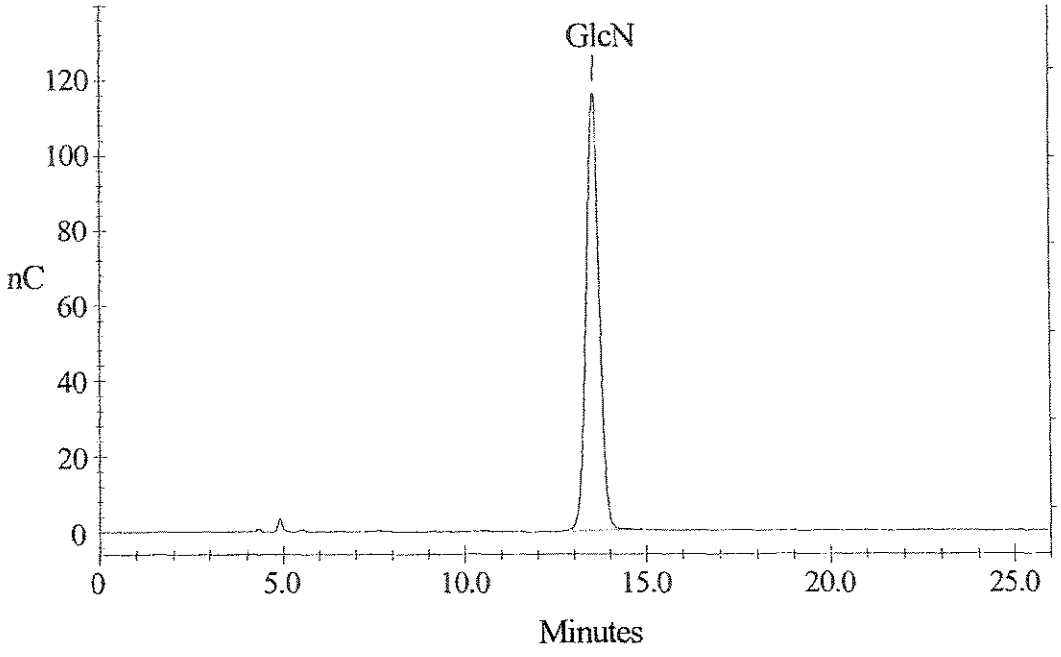



FIG. 3

# GLUCOSAMINE AND METHOD OF MAKING GLUCOSAMINE FROM MICROBIAL BIOMASS

## PRIORITY CLAIM

This application is a continuation of pending U.S. patent application Ser. No. 09/785,695, filed Feb. 16, 2001 now abandoned.

## FIELD

The present invention is directed to glucosamine compositions and to methods of making glucosamine compositions.

## BACKGROUND

Glucosamine is a nutraceutical supplement that has been shown to provide significant therapeutic relief for arthritis and joint pain. Although the mechanism is not entirely known, it is believed that glucosamine functions to aid in restoration of the cartilage to relieve inflammation in the joints, thereby providing significant benefit to patients.

Presently, glucosamine is primarily derived from harvested natural sources, such as shellfish and other aquatic organisms. Components of the shell or exoskeleton of these organisms are converted into glucosamine using various production techniques. These natural sources are acceptable for producing glucosamine for some applications, but they have limitations. These limitations include the fact that wild shellfish can have significant variations in their composition because they grow naturally under uncontrolled circumstances. The shellfish can vary in such aspects as their size and composition depending upon the growing conditions as well as their species. Also, without control over the growing conditions, the shellfish can be exposed to environmental contaminants, including heavy metals, that can be retained in glucosamine or other products produced from the shellfish. Shellfish harvests are often seasonal, and thus the supply and price of shellfish shows significant variation over time.

A further concern with glucosamine derived from shellfish is that significant portions of the human population have shellfish allergies and are unable to use products that contain ingredients derived from shellfish. Highly processed materials, such as glucosamine, do not necessarily provide any allergic risk when prepared properly; but a concern remains that hyper allergenic individuals will still be allergic to even minute traces of allergens present from the original shellfish. Even if no such allergens are present, glucosamine derived from shellfish can pose a concern to individuals who are allergic to shellfish because individual consumers are not necessarily aware of whether or not all of the allergens have been removed.

An additional problem associated with existing sources of shellfish-derived glucosamine is that some of the shellfish supply is harvested from the seas and oceans of the world. Excessive harvest of shellfish could have a great negative environmental impact. Thus, it is believed that some consumers would prefer to use glucosamine that is not harvested at the expense of sea life. Even if the environmental impact of harvesting shellfish is not negative, there remains concern that the supply of wild shellfish is limited in quantity and inconsistent in quantity from year to year.

Therefore, a need exists for a source of safe, consistent, high quality glucosamine that can be created economically and with a minimum of environmental impact.



## SUMMARY

The present invention is directed to glucosamine, including glucosamine-containing material suitable for human or animal consumption. Glucosamine of the present invention is derived from fermented fungal biomass containing chitin. Suitable starting materials include substantially uniform microbial fungal sources, such as fungal sources derived from *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., and combinations thereof. Use of a fungal biomass results in a high quality product that produces generally uniform glucosamine having low levels of impurities. The glucosamine of the present invention normally has relatively low ash content, and low heavy metal content. In addition, as a product of fungal biomass, the glucosamine does not pose a hazard to persons who have shellfish allergies.

The present invention is also directed to methods of producing glucosamine by acid hydrolysis of fermented fungal biomass. The methods of obtaining glucosamine from microbial biomass include reacting chitin-containing biomass in an acidic solution, in particular reacting the chitin-containing biomass in acid at an elevated temperature.

Other features and advantages of the invention will be apparent from the following detailed description of the invention and the claims. The above summary of principles of the disclosure is not intended to describe each illustrated embodiment or every implementation of the present disclosure. The detailed description that follows more particularly exemplifies certain embodiments utilizing the principles disclosed herein.

## DRAWINGS

The invention will be more fully explained with reference to the following drawings, in which:

FIG. **1** is chart showing the percent yield of glucosamine over time of an example method of making glucosamine in accordance with the invention.

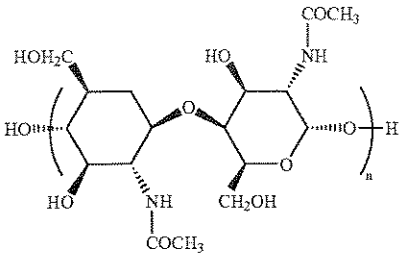
FIG. **2** is a chromatogram of glucosamine made in accordance with the invention.

FIG. **3** is a chromatogram of glucosamine made in accordance with the invention.

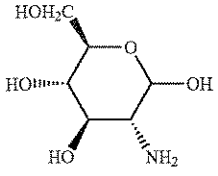
While principles of the invention are amenable to various modifications and alternative forms, specifics thereof have been shown by way of example and will be described in detail. It should be understood, however, that the intention is not to limit the invention to the particular embodiments described. On the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the disclosure.

## DETAILED DESCRIPTION

The present invention is directed to glucosamine, including glucosamine-containing material suitable for human or animal consumption. The glucosamine is derived from chitin present in various types of fungal biomass. Chitin is a natural polysaccharide, with the structure of an unbranched polymer of 2-acetoamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine). This formula can be represented by the general repeating structure:



Chitin is typically an amorphous solid that is largely insoluble in water, dilute acids, and alkali. Although chitin has various commercial applications, greater commercial utility can be found by transforming the polymeric structure into individual components of 2-amino-2-deoxy-D-glucose, which is known as glucosamine. Structurally, glucosamine is modified glucose with an amine group replacing the OH group found on carbon two (C-2). The general structure is:



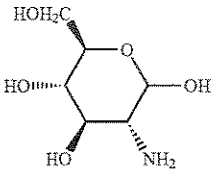
As stated above, glucosamine of the present invention is derived from fermented fungal biomass containing chitin. Suitable starting materials include substantially uniform microbial fungal sources, such as fungal sources derived from *Aspergillus* sp., *Penicillium* sp., *Mucor* sp. and combinations thereof. Use of a fungal biomass results in a high quality product that produces a generally uniform glucosamine having low levels of impurities. The glucosamine of the present invention normally has relatively low ash content, and low heavy metals content. In addition, as a product of fungal biomass, the glucosamine does not pose a hazard to persons who have shellfish allergies.

The glucosamine composition, starting materials, and production methods will now be described in greater detail

A. Glucosamine

The glucosamine of the present invention is derived from relatively uniform fungal biomass sources, and thus typically has a generally uniform composition. Depending upon the methodology used to purify the glucosamine or desired glucosamine salt; the resulting glucosamine containing composition can be produced with varying levels of purity, including compositions that exceed 95 percent purity, 98 percent purity, and even 99.8 percent purity. The glucosamine compositions can also contain additional ingredients, such as additional salts. In such circumstances the overall purity of the desired composition relative to undesirable impurities can be maintained at levels that exceed 95 percent purity, 98 percent purity, and even 99.8 percent purity.

The glucosamine of the present invention has the general formula represented below:



This general formula can vary depending upon the presence of various salts of the glucosamine, including citrate, acetate, phosphate, sulfate, chloride, lactate, gluconate, etc. Also, the glucosamine can be substituted or modified without diverging from the scope of the invention. Thus, as used herein, the term glucosamine refers to the various forms of glucosamine, including salt complexes and substituted glucosamine.

The glucosamine is normally of high purity, but can contain other ingredients, including glucose, unreacted chitin, and other materials. Preferably the glucosamine contains less than 10 percent glucose, more preferably less than 5 percent glucose, and even more preferably less than 2 percent glucose.

The glucosamine of the present invention has a relatively low ash content. The ash content is usually less than 5 percent, more typically less than 2 percent, and can even be less than 1 percent in some implementations. Heavy metal content is normally similarly low, typically well below 100 parts per million, more typically below 50 parts per million, even more typically below 20 parts per million. In certain embodiments this level is below 10 parts per million. The glucosamine can have a positive specific rotation, such as a positive 69 to 74 degree specific rotation for the glucosamine hydrochloride salt.

The glucosamine of the invention is usually relatively white in its purified dry form, but colorless when dissolved in an aqueous solution. In one example, a 20 percent by weight solution of the glucosamine has an American Public Health Association (APHA) color of less than 50.

B. Microbial Fungal Biomass Starting Materials

Suitable starting materials include substantially uniform microbial biomass sources, typically fungal biomass, such as filamentous fungi having greater than 10 percent chitin by total dry cell weight, such as fungal sources derived from *Aspergillus* sp., *Penicillium* sp., *Mucor* sp. Suitable fungal biomasses include *Aspergillus niger, Aspergillus terreus, Aspergillus oryzae, Mucor rouxii, Penicillium chrysogenum, Penicillium notatum, Saccharomyces cerevisiae; Saccharomyces uvarum*; and in particular *Candida guillermondi, Aspergillus niger*, and *Aspergillus terreus*. The biomass is usually recovered from a commercial fermentation reaction, such as the commercial production of organic acids, including citric acid. Also, the biomass suitable for production of glucosamine can be generated specifically for this process and not as a byproduct of other processes. As used herein, the term microbial does not include phyto-plankton and crustaceans or mollusks.

The invention is particularly well suited to uses where the chitin levels in the biomass exceed 5 percent of the dry biomass weight. Such biomass usually has between 5 and 25 percent chitin, and can have from 10 to 20 percent chitin, based upon dry weight of the biomass. Also, in order to prepare the highest quality glucosamine, it is sometimes desirable that the microbial biomass be produced in a

substantially controlled manner having relatively uniform temperature and nutrient levels during the growth of the biomass.

C. Glucosamine Production Methods

The present invention is also directed to methods of forming glucosamine, including formation from acid hydrolysis of fermented fungal biomass. The acid hydrolysis breaks the ether linkages and deacetylates the chitin molecule to generate free glucosamine. Acid hydrolysis is strong enough to break the chitin into glucosamine, but leaves the glucosamine molecule substantially intact. The hydrolysis reaction conditions have the added advantage of breaking down some of the other components (such glucans, proteins, and lipids) that exist in the fungal biomass. Typically, such acid hydrolysis is performed by treating the fungal biomass for greater than 4 hours in a strong acid solution.

Glucosamine production usually includes the steps of providing chitin-containing biomass, reacting the chitin-containing biomass in an acidic solution to form glucosamine, and separating the glucosamine from the acidic solution. The reaction typically has a yield of glucosamine of greater than 50 percent of total chitin content of the fungal biomass starting material.

Strong acids can be used to hydrolyze the fungal biomass, including acids of concentrations less than 50 percent, and more commonly from 5 to 25 percent. Suitable strong acids include hydrochloric, sulfuric, phosphoric, and citric acid at appropriate levels.

The glucosamine forming reaction is normally conducted with 5 to 20 percent acid, 2 to 50 percent pretreated biomass (based upon dry weight, although the biomass is typically processed with water present), and 35 to 93 percent water. In certain implementations the reaction mixture comprises from 8 to 12 percent hydrochloric acid, from 4 to 8 percent biomass (based upon dry weight), and from 80 to 90 percent water.

The mixture containing the biomass, acid, and water is heated and maintained at an elevated temperature. The mixture is usually heated to a temperature at or near its boiling point and maintained under reflux conditions for greater than 5 hours, more typically greater than 8 hours, and usually less than 16 hours. It is desirable to have the reaction continue long enough to have a complete breakdown of the chitin, but not take so long as to be inefficient or to excessively decompose the glucosamine.

Reaction in the acid solution produces glucosamine, but subsequent purification steps are typically necessary to produce a satisfactory product. A first purification step normally includes filtration to remove particulate impurities, resulting in a substantially clear filtrate. This filtrate normally contains glucosamine, as well as small quantities of glucose and other sugars. An evaporative step can subsequently be performed to concentrate the glucosamine and possibly remove some of the acid, which can be recycled and reused. The mixture can be concentrated by evaporation, and the glucosamine can be precipitated out as purified solids by either adding ethanol to the concentrated mixture or continuing the evaporation to its solubility limits.

The glucosamine can be recovered by filtration or centrifugation, followed by drying. The dried glucosamine is optionally further purified to remove any residual sugar. One method of removing these excess sugars is by dissolving the glucosamine in water and adding ethanol, which precipitates the glucosamine at greater purity. Alternatively, the solution can be purified by electro dialysis, chromatography, membrane filtration, etc. The glucosamine is optionally decolorized with ethanol, carbon, or other suitable material and method.

In addition to the steps described above, the biomass can initially be treated to remove some impurities or to improve glucosamine production. These treatments can include heating the biomass, adding digestive enzymes, mixing with an acid or base, mechanical agitation, or dewatering by compression. One particularly suitable treatment is pretreating the biomass in the presence of sodium hydroxide. In certain implementations a concentration of less than 10 percent sodium hydroxide is added to the fungal biomass, which is heated to an elevated temperature for a period sufficient to remove a considerable portion of the non-chitin containing material. This period is normally less than two hours. One specific example of this pretreatment method requires heating the fungal biomass to 100 to 125° C. in a 2 to 8 percent solution of sodium hydroxide for 20 to 60 minutes. This step hydrolyzes some protein and glucan in the biomass, the byproducts of which are optionally removed by filtration. The filtration step is followed to remove soluble proteins, amino acids, etc. In specific implementations of the invention, the washed and pretreated biomass contains greater than 50 percent water, and even greater than 70 or 80 percent water. Typically the water level is from about 80 to 95 percent for this prewashed fungal biomass.

D. EXAMPLES

The invention will be further explained by the following non-limiting illustrative examples. Unless otherwise indicated, all amounts are expressed in parts by weight.

Example 1

Citric biomass was pretreated with a 4 percent aqueous sodium hydroxide (NaOH) solution in an autoclave at 120° C. for 1 hour. This step removed excess proteins and other undesirable materials. The biomass was then thoroughly washed with de-ionized water until its pH was approximately 7.0. This washed material was mixed with concentrated hydrochloric acid (HCl) and water to form a mixture of 10 to 15 percent HCl and 5 to 6 percent biomass, based upon dry weight of the biomass. This mixture was heated at reflux. Samples were taken from time to time, and the reaction analyzed with a high-pressure liquid chromatograph available from Dionex HPLC under the trade designation "DX-500".

The results are provided in FIG. **1**, which shows a chart indicating glucosamine production, and shows that the glucosamine was increasingly produced as the reaction ran through 8 hours, but that the amount of glucose diminished after 4 hours. After 8 hours the glucosamine produced in the yield of 14 percent. A chromatogram of the product is shown in FIG. **2**.

Following reaction, the mixture was filtered, and the filtrate evaporated using a rotating evaporator manufactured by RotaVap to increase the glucosamine concentration of the solution. The final volume was reduced to about 10 to 20 ml. To this solution was added 20 ml of ethanol and the solution swirled to promote precipitation of glucosamine and enhance yield. These glucosamine precipitates were obtained by filtration and were further washed with alcohol until the color became white. FIG. **3** shows a chromatogram of the product, indicating greater than 97 percent glucosamine.

Example 2

Example 1 was repeated, but the pretreated biomass was maintained under reflux conditions for 13 hours. The resulting glucosamine was greater than 98 percent pure.

The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood from this description or examples. The invention is not limited to the exact details shown and described, for variations will be included within the invention defined by the claims.

We claim:

**1**. A method of obtaining glucosamine from fungal biomass, the method comprising the steps of:

(a) providing the fungal biomass;

(b) reacting the fungal biomass in an acidic solution with an acid concentration of greater than 5 percent by weight at a reaction temperature greater than 80° C. for a reaction period of at least 4 hours to convert chitin in the fungal biomass to glucosamine; and

(c) separating the glucosamine from the acidic solution;

wherein the method has a yield of glucosamine of greater than 50% of total chitin content of the fungal biomass.

**2**. The method of claim **1**, wherein the step of separating the glucosamine comprises crystallization of the glucosamine from the acidic solution.

**3**. The method of claim **1**, wherein the acid solution has an acid concentration of 5 to 25 percent by weight.

**4**. The method of claim **1**, wherein the acid solution has an acid concentration of 5 to 50 percent by weight.

**5**. The method of claim **1**, wherein the reaction temperature is above 80° C.

**6**. The method of claim **1**, wherein the reaction period is from 4 to 25 hours.

**7**. The method of claim **1**, wherein the separated glucosamine has a yield of greater than 50 percent of total chitin content of the fungal biomass and contains less than 5 percent glucose.

**8**. The method of claim **1**, wherein the separated glucosamine contains less than 5 percent soluble sugars.

**9**. The method of claim **1**, wherein the separated glucosamine is greater than 98 percent glucosamine based upon dry weight.

**10**. The method of claim **1**, wherein the glucosamine is derived from *Aspergillus* sp., *Penicillium* sp., *Mucor* sp. and combinations thereof.

**11**. A method of obtaining glucosamine from fungal biomass, the method comprising the steps of:

(a) providing fungal biomass;

(b) pretreating the fungal biomass with an alkaline solution;

(c) reacting the fungal biomass in an acidic solution with an acid concentration of greater than 5 percent by weight at a reaction temperature greater than 80° C. for a reaction period of at least 4 hours to convert chitin in the fungal biomass to glucosamine; and

(d) separating the glucosamine from the acidic solution;

wherein the method has a yield glucosamine of greater than 50% of total chitin content of the fungal biomass.

**12**. The method of claim **11**, wherein greater than 50 percent of total chitin content of the fungal biomass is converted to glucosamine and the glucosamine separated from the acidic solution contains less than 5 percent glucose.

**13**. The method of claim **11**, wherein the glucosamine separated from the acidic solution contains less than 20 parts per million heavy metals.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION



PATENT NO. : 7,049,433 B2
APPLICATION NO. : 10/326549
DATED : May 23, 2006
INVENTOR(S) : Weiyu Fan et al.

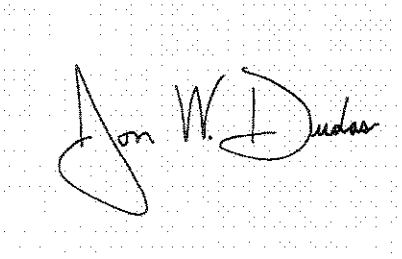
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims:

Column 8, line 24, "yield glucosamine" should be --yield of glucosamine--.

Signed and Sealed this

Sixth Day of November, 2007



JON W. DUDAS
*Director of the United States Patent and Trademark Office*

VERIFICATION OF COMPLAINT

I, Jack Staloch, am Vice President of Cargill Corn Milling North America, Director BioTDC, for Cargill, Incorporated ("Cargill") and am duly authorized to execute this complaint on behalf of Cargill. I have read the complaint and am aware of its contents. To the best of my knowledge, information, and belief, formed after an inquiry reasonable under the circumstances, I hereby certify as follows:

1. The complaint is not being presented for any improper purpose, such as to harass or cause unnecessary delay or needless increase in the cost of the investigation;

2. The claims and other legal contentions in the complaint are warranted by existing law or by a nonfrivolous argument for the extension, modification, or reversal of existing law or the establishment of new law; and

3. The allegations and other factual contentions in the complaint have evidentiary support or, if specifically so identified, are likely to have evidentiary support after a reasonable opportunity for further investigation or discovery.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on January 27, 2009.

Jack Staloch

Jack Staloch
Vice President of Cargill Corn Milling North America
Director BioTDC

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(212) 506-2645
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*Attorney for the Plaintiff*

**IN THE UNITED STATES DISTRICT COURT**
**FOR THE DISTRICT OF NEW JERSEY**

| | | |
|---|---|---|
| CARGILL, INCORPORATED | ) | |
| | ) | |
| Plaintiff, | ) | |
| | ) | |
| v. | ) | Civil Action No. |
| | ) | |
| NANTONG FOREIGN TRADE MEDICINES AND HEALTH PRODUCTS CO., LTD., DNP INTERNATIONAL, INC., TIANCHENG INTERNATIONAL, INC. (USA), HYGIEIA HEALTH CO., LTD., TSI HEALTH SCIENCES, INC. (USA), and ETHICAL NATURALS, INC. | ) | |
| | ) | |
| Defendants. | ) | |

**CERTIFICATION**

The matter in controversy is the subject of an action filed on January 28, 2009 in the United States International Trade Commission ("ITC Action"). Except for the ITC Action, the matter in controversy is not the subject of any other action pending in any court, or of any pending arbitration or administrative proceeding.

Dated: January 28, 2009

Respectfully submitted,

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